AUTOMATIC MULTIPLE-DECANTING CENTRIFUGE

CROSS REFERENCE TO RELATED APPLICATION

This application is a division of U.S. application Ser. No. 08/435,662, which was filed on May 5, 1995, now U.S. Pat. No. 5,707,331.

TECHNICAL FIELD

This invention relates to the art of automatic centrifugation. In particular, the invention relates to apparatus and procedures using automatic, multiple decanting with centrifugation. In a preferred embodiment, an automated procedure separates blood components and proteins including the separation of fibrinogen from blood.

BACKGROUND

The separation of components through centrifugation is well known. For example, in the medical field it is common to subject a sample of blood to centrifugation to produce a precipitate of cellular material and a supernatant of plasma. The plasma is then decanted to complete the separation of these components.

U.S. Pat. Nos. 5,178,602 (Wells) and 5,047,004 (Wells) show an automated centrifuge, which includes structure for holding a centrifuge tube, after centrifugation, in a position that allows the supernatant to drain from the tube and into another container by gravity. The holding structure shown in these patents comprises a locking mechanism mounted for axial movement with respect to the axis of rotation of the centrifuge. An electromagnet that is easily controlled causes the axial movement.

It is also known to decant a supernatant by the process of centrifugal draining. According to that process, a centrifuge rotates a centrifuge tube while the tube is held in a position such that the supernatant is drained from the tube by centrifugal forces.

Fibrin sealants for treating wounds are known and are typically produced by combining a fibrinogen/Factor XIII component with bovine thrombin. When these are mixed, a fibrin tissue adhesive results, which is applied to the wound. Descriptions of compositions for use as tissue sealants are given in U.S. Pat. Nos. 5,292,362 and 5,209,776 (Bass et al.). The fibrinogen is obtained from plasma, either pooled or autologous, and cryoprecipitation is one known technique for separating fibrinogen from plasma. One cryoprecipitation technique is described in U.S. Pat. Nos. 5,318,524 and includes the centrifugation of thawing plasma to produce a precipitate containing fibrinogen/Factor XIII. Other techniques for producing fibrinogen/Factor XIII include inducing precipitation of the component by addition of such agents as Ammonium Sulfate or polyethylene glycol (PEG) to blood plasma.

SUMMARY OF THE INVENTION

Several known chemical procedures include repeated steps of physical separation between two or more components. Separation based on density differences between the components is often by centrifugation, and the resulting supernatant is decanted to complete the separation. Each step provides an opportunity for error, which would be reduced by automation of the process.

In accordance with the invention, chemical procedures requiring several centrifugation steps are automated, to reduce the time required by a clinician and eliminate the potential for errors. Apparatus in accordance with the invention includes a multiple-chamber container and a centrifuge designed to receive the container and subject its contents to predetermined centrifugation steps as well as gravity and centrifugal decanting of the supernatant.

A preferred container in accordance with the invention includes first and second chambers separated by an intermediate wall. The first chamber is designed to receive a first liquid, such as human blood. The second chamber is located adjacent the first chamber, and the wall between the chambers is such that a supernatant in the first chamber will flow over the top of the wall and be drained into the second chamber by gravity when the container is held in the proper orientation. The supernatant in the second chamber may then be subjected to a mixing action and then may be subjected to a second centrifugation. The container can also be held in a second position whereby a second supernatant is caused to flow back over the wall into the first chamber by centrifugal forces resulting from a second centrifugation.

A centrifuge in accordance with the invention includes a rotatable support with a swinging frame for receiving the multiple-chamber container and means for locking the container in either of at least two positions for draining supernatant fluids from the chambers. Preferably, the locking means is an electro-magnetically operated disk mounted for movement axially with respect to the axis of rotation of the rotatable support. The centrifuge is preferably operated under the control of an electronic circuit, which may include a programmed array logic (PAL) or other circuitry, that causes the rotor to operate in accordance with a predetermined program and controls the locking means such that it locks the container in predetermined orientations in conjunction with operation of the rotor.

While many different programs for operation of the centrifuge can be developed, depending on the desired results, a preferred operation is for the production of autologous fibrinogen. Prior techniques for production of fibrinogen require several distinct steps, each of which requires a skilled technician but does not eliminate an opportunity for error. These steps include separation of plasma from cellular components, treatment of the plasma with a precipitating agent, and separation of a fibrinogen precipitate "pellet" from the plasma. The separation of plasma from blood and the separation of the fibrinogen pellet from plasma typically require centrifugation first of the blood and then of the plasma, with addition of at least one precipitating agent between the steps. Thus, the production of fibrinogen in the prior art has been complex and error-prone.

In accordance with this embodiment of the invention, a volume of the patient's anticoagulated blood is placed in the first chamber of the disposable container, and a precipitation agent is placed in the second of the chambers. The container is then placed in the swinging frame of the centrifuge, and the control circuit is activated to initiate the operation of the centrifuge. The centrifuge first rotates the container for a time period that has been determined to be adequate for separating the cellular components from the supernatant plasma. During this time, the swinging frame will have rotated outwardly substantially due to centrifugal forces on the container. While the frame is in the outwardly rotated position, the locking means is activated to lock it there. The rotation of the support is then terminated. As the rotational velocity of the support decreases, the supernatant fluid, being no longer subject to the centrifugal forces, flows out of the first chamber and into the second chamber by gravity. The cellular component is more viscous and, thus, flows

toward the second chamber at a rate less than that of the plasma.. Preferably, however, a divider in the form of a disk is placed in the first chamber to restrict the flow of the cellular components and plasma below the disk. The disk is at a depth that provides a predetermined volume of plasma, which is normally near the expected boundary between the supernatant and cellular components. After a period of time that has been determined to allow an adequate amount of the plasma to flow into the second chamber, the locking means is deactivated to release the container, whereby it assumes an upright position with the cellular component remaining in the first chamber and the plasma now in the second chamber. The rotatable support is then alternately activated and deactivated for short intervals to mix the plasma with the precipitating agent in the second chamber. Interaction between the precipitating agent and the plasma initiates precipitation of fibrinogen and Factor XIII from the plasma. The support is then again rotated to accelerate the precipitation of the fibrinogen/Factor XIII and to create a pellet in the bottom of the second chamber. As a final step, the locking means is again activated to lock the container in a position such that the supernatant resulting from precipitation of the fibrinogen is decanted by centrifugal draining into the first chamber. In this step, the container is held substantially upright, and the support is rotated to apply centrifugal forces to the supernatant, whereby it flows over the wall between the chambers and into the first chamber. The locking means is then inactivated, the container removed from the centrifuge, and the fibrinogen/Factor XIII removed from the second chamber for further processing. In a preferred embodiment, the fibrinogen/Factor XIII is reconstituted, and then combined with thrombin, and applied to a patient to treat a wound.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a perspective of a container and centrifuge in accordance with the invention.

FIG. **2** is a vertical cross section of a preferred embodiment of a container.

FIGS. **3***a* and **3***b* are partial vertical cross sections of the centrifuge of FIG. **1**.

FIGS. **4***a* through **4***f* are schematic diagrams illustrating a preferred method of operation of the centrifuge of the invention.

DETAILED DESCRIPTION OF THE INVENTION

With reference to FIGS. **1** and **2** of the drawings, a centrifuge **2** is designed to receive a container **4** in accordance with the invention. The centrifuge is capable of subjecting the container to a series of steps that will be described in detail below. The container includes at least two chambers, **6** and **8**. Chamber **6** is designed to receive a first fluid to be treated, such as blood. Chamber **8** is designed to receive fluids that have been decanted from chamber **6**, such as a supernatant plasma resulting from centrifugation of blood in chamber **6**.

A preferred form of the container is shown in detail in FIG. **2**. As shown, the container comprises three primary parts. A base part is preferably molded and includes the chambers **6** and **8** and a bridge **7**, which connects the two chambers. A lid **11**, also preferably molded, fits over the tops of the chambers to close them. The lid includes cup shaped extensions **12** and **14**, each of which is centrally aligned with a respective one of the chambers **6** and **8**. Extension **12** has a access port in the form of centrally located opening **13**, while extension **14** has a centrally located opening **15**. The openings receive syringe needles to permit fluids to be injected into the chambers or withdrawn therefrom. Membranes **16** and **17** cover the openings **13** and **15** to maintain sterility. The membranes are preferably heat sealed into the extensions **12** and **14** during construction by providing a cavity for receiving the membranes. After a membrane is inserted, the upper edges of the cavity are folded over and welded, e.g., ultrasonically, to retain the membrane.

The lid also includes a bridge **7'** that cooperates with bridge **7** in the base to form a fluid channel **18**, connecting chambers **6** and **8**. As shown, the bridge **7** extends above the tops of the chambers **6** and **8** to prevent communication between the chambers by "splashing." Intentional fluid communication between the two chambers will be described in detail below.

A separation disk **20** is preferably placed in chamber **6** near, but always above, the expected vertical position of the boundary between supernatant plasma and cellular components after a first centrifugation of a blood sample. The hematocrit is known to vary among individuals, and the exact amount of plasma that will result from a blood sample cannot be accurately predicted without prior testing of the sample. Thus, disk **20** is located such that the plasma above the disk after centrifugation of a predetermined volume of blood is a predetermined volume of plasma. The upper surface of the disk **20** is tapered toward an edge, and the edge includes at least one groove **22** that allows fluid communication between the parts of the chamber **6** that are above and below the disk **20**.

In a preferred embodiment, a cylindrical support **24** is attached to the lower surface of the disk to set the location of the disk during assembly.

A hollow tube **26** is provided to facilitate introduction of the blood sample to the portion of the chamber **6** that is below the disk **20**. The tube **26** extends from just below the opening **13** through disk **20**. Thus, a syringe needle inserted through opening **13** pierces membrane **16** and communicates with tube **26** to allow injection of the blood sample into the bottom of the chamber **6**. The groove **22** permits downward movement of the plasma and cellular components during centrifugation but retards movement of the cellular components during decanting. Also, an air vent **27** is provided for chamber **8** to facilitate introduction and withdrawal of fluids.

In use, a container **4** is placed in a holder on the rotor of the centrifuge as indicated in FIG. **1**. To balance the rotor, two such containers are preferably placed in the centrifuge in diametrically opposed positions. Of course, only one container may be used and a weight or "dummy" container used to balance the rotor.

FIGS. **3***a* and **3***b* are partial cross sections of a preferred embodiment of a centrifuge showing the container locked in two different positions. A rotor shaft **28** is connected to a motor (not shown), which rotates the shaft. A rotor **30** is mounted to the shaft for rotation and has a frame **32** pivotally mounted to the rotor **30** at pivot connection **34**. The top surface (not shown) of the frame **32** has two circular openings for receiving the chambers **6** and **8** whereby the container can be placed in the frame such that the contents of the container will be subjected to centrifugal forces as the rotor is rotated. A bias spring **35** ensures that the frame **32** will pivot to an upright position when centrifugation is terminated. The frame **32** may also be shaped to reduce wind resistance, as known in the art.

A locking plate **36** is mounted coaxially with the shaft **28** for engaging the frame **32** to lock the container in desired